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**Addendum to the  
Toxicological Review of Trichloroethylene**

[CASRN 79-01-6]

**In Support of Summary Information on the  
Integrated Risk Information System (IRIS):**

**Comparing Less-than-Chronic Exposure Concentration Estimates with  
the Reference Concentration**

*September 2013*

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National Center for Environmental Assessment  
Office of Research and Development  
U.S. Environmental Protection Agency  
Washington, DC

***Addendum to the Toxicological Review of Trichloroethylene:  
Comparing Less-than-Chronic Exposure Concentration Estimates with the RfC***

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**ABBREVIATIONS**

|                   |  |      |  |
|-------------------|--|------|--|
| CASRN             | Chemical Abstracts Service Registry Number | NCEA | National Center for Environmental Assessment |
| EPA               | Environmental Protection Agency            | NRC  | National Research Council                    |
| EC                | exposure concentration                     | ORD  | Office of Research and Development           |
| HEC               | human equivalent concentration             | PBPK | physiologically based pharmacokinetic        |
| HQ                | hazard quotient                            | RfC  | inhalation reference concentration           |
| IOM               | Institute of Medicine                      | RfV  | reference value                              |
| IRIS              | Integrated Risk Information System         | SAB  | Science Advisory Board                       |
| LMP               | last menstrual period                      | TCA  | trichloroacetic acid                         |
| µg/m <sup>3</sup> | microgram per cubic meter                  | TCE  | trichloroethylene                            |
|                   |  | TK   | toxicokinetics                               |
|                   |  | U.S. | United States of America                     |

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1 A public meeting was held by EPA on [month] [date], [year]. Attendees external to the EPA are  
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3 This addendum was released for public comment on [month] [day], [year] and comments were due  
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5 This addendum was peer reviewed by independent expert scientists external to EPA and a peer-  
6 review meeting was held on [month] [day], [year]. The external peer review comments are  
7 available on the IRIS Web site. A summary and EPA’s disposition of the comments received from  
8 the independent external peer reviewers and from the public is included in Appendix [X] and is also  
9 available on the IRIS Web site.

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## **PREFACE**

In September 2011, EPA posted the IRIS Summary for Trichloroethylene (TCE) (CASRN 79-01-6) and the corresponding *Toxicological Review of Trichloroethylene in Support of the Integrated Risk Information System* ("IRIS Toxicological Review of TCE") (U.S. EPA, 2011). The IRIS Toxicological Review of TCE includes a reference concentration (RfC), which is defined as the estimated continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious [noncancer] effects during a lifetime (U.S. EPA, 2002). The RfC (2 µg/m<sup>3</sup>) for TCE is based on two health effects: the adult immunotoxicity endpoint of decreased thymus weights in mice (Keil et al., 2009) and the developmental toxicity endpoint of increased incidence of fetal cardiac malformations in rats (Johnson et al., 2003). The RfC is derived in the context of continuous exposure at a constant level over a chronic duration. Because exposed populations generally include individuals of different genders and ages, at any given point in time, there may be exposures occurring during pre- and post-natal development in which there may be windows of susceptibility to developmental toxicity. Therefore, it is appropriate to base the RfC wholly or in part on a developmental effect. Because windows of susceptibility for human developmental effects are less-than-chronic in duration, developmental effects have also been used to derive shorter-than-chronic reference values, including acute (24-hour) reference values (Solecki et al., 2010; U.S. EPA, 2002). However, reference values for less-than-chronic exposure scenarios were not explicitly developed as part of the IRIS Toxicological Review of TCE.

Inhalation reference values (RfVs) such as the RfC may be combined with an exposure concentration (EC) to obtain a hazard quotient (HQ) (where  $HQ = EC/RfV$ ) to characterize risk, where ECs are defined as time-weighted average concentrations over a specified exposure duration, and are derived from measured or modeled contaminant concentrations in air. Because air concentrations of TCE, such as indoor air concentrations as a result of TCE vapor intrusion,<sup>1</sup> can fluctuate significantly over time, different exposure durations can lead to different estimated ECs. The IRIS RfC for TCE is based, in part, on a malformation in an organ for which there is a period of morphogenic development during which it is susceptible. Therefore, even if average exposures

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<sup>1</sup> Vapor intrusion generally occurs when there is a migration of volatile chemicals from contaminated groundwater or soil into an overlying building. Volatile chemicals can emit vapors that may migrate through subsurface soils and into indoor air spaces of overlying buildings in ways similar to that of radon gas seeping into homes. See <http://www.epa.gov/oswer/vaporintrusion/> for more information.

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over a longer duration are without appreciable risk (e.g., at or below the RfC), fluctuations leading to higher levels during shorter periods may be of concern.

The use of developmental effects for characterizing risk from exposure durations as short as one day is an established EPA practice. The application of this practice recognizes the following generic considerations (U.S. EPA, 2002, 1991):

- “Site concordance” is generally not assumed for developmental effects, so a particular developmental endpoint observed in an experimental animal model is often used to infer general developmental toxicity in humans.<sup>2</sup>
- It is a well-established fact that developmental toxicity may result from even a single exposure during a developmental window.
- The derivation of a shorter-than-chronic reference value should consider toxicokinetics, particularly if there is a potential for bioaccumulation.

Based on these generic considerations, the TCE RfC is relevant to exposures of less-than-chronic duration, including single day exposures. However, in some cases, the selection of appropriate exposure durations for comparability to the RfC may be based on chemical-specific information regarding the developmental endpoint of concern and the toxicokinetics (TK) pertaining to that specific chemical and endpoint.

This Addendum discusses how the available data on TCE, cardiac malformations, and cardiac development may inform the appropriate duration for derivation of ECs for comparison with the IRIS TCE RfC.<sup>3</sup> Therefore, the goal of this Addendum is to estimate the “exposure duration for comparability to the RfC,” which is defined as the exposure duration over which ECs may be averaged that would yield a characterization of risk comparable to that estimated for a constant, continuous, chronic exposure scenario. Because time-weighted averaging leads to “smoothing” over fluctuations in exposure levels over the specified duration while also excluding consideration of exposures outside of the specified duration, determining what exposure duration is most comparable to a constant, continuous, chronic exposure scenario will depend on a number of different considerations, summarized in the following questions:

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<sup>2</sup> “Site concordance” is defined here as the same type(s) of effect(s) occurring in the same target tissue as a result of toxicant exposure in different species.

<sup>3</sup> A number of additional developmental effects have also been associated with TCE, and a similar analysis on exposure durations could be performed for each developmental endpoint. However, this Addendum focuses only on the endpoint of cardiac malformations because it was the only developmental endpoint upon which the IRIS TCE RfC was based. It should also be noted that the candidate RfCs developed for other developmental effects are more than an order of magnitude higher (between 30- and 6000-fold) than the overall RfC (U.S. EPA, 2011).

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1) Are the data on TCE and cardiac malformations adequate to infer a site-concordant hazard from TCE specifically for this developmental endpoint rather than a general developmental toxicity hazard?

2) What is the most appropriate exposure duration for comparability to the RfC for TCE-induced cardiac malformations?

This question incorporates the following factors:

a. the duration of the window of susceptibility for TCE-induced cardiac malformations in humans;

b. the extent to which the response depends on cumulative internal dose integrated over the window of susceptibility; and

c. the toxicokinetics related to potential bioaccumulation of internal dose.

The first question is addressed in Chapter 1, and the second question in Chapter 2. The conclusions are summarized in Chapter 3.

# 1. TCE-INDUCED CARDIAC MALFORMATIONS AND SITE CONCORDANCE

## 1.1. Discussion

The hazard evaluation supporting the use of cardiac malformations as a basis for the RfC takes into consideration the epidemiologic, rodent, avian, and in vitro data on this endpoint and reflects an evaluation of cardiac teratogenicity specifically rather than developmental toxicity in general.<sup>4</sup> Thus, the data on TCE and cardiac malformations support an inference of site-concordant hazard from TCE for this developmental endpoint rather than a default inference of general developmental toxicity. These data and conclusions are summarized as follows.

The cardiac teratogenicity of TCE has been the focus of considerable study and analysis. The National Research Council (NRC), in their report *Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues*, noted that the epidemiological studies, although individually limited, as a whole show relatively consistent elevations for cardiac malformations, with similar relative effect sizes of two- to threefold, some of which were statistically significant, associated with TCE exposure across multiple studies (NRC, 2006). These epidemiologic studies are geographically based, and interpretation of these data has been controversial, since many of the studies have small numbers of cases, insufficient exposure characterization, chemical co-exposures, low statistical power, and other methodological deficiencies, with uncertainties regarding the lack of information on exposure potential and exposure level for individual subjects, and inability to completely adjust for other potential risk factors (Forand et al., 2012; ATSDR, 2008, 2006; Yauck et al., 2004; Bove, 1996; Bove et al., 1995; Goldberg et al., 1990). Moreover, while these studies involved exposures to the general population via drinking water or indoor air, none were suitable for dose-response assessment.

The outcomes of studies in rodents exposed to TCE during gestation show an inconsistent pattern. Some studies identified significant treatment-related increases in the overall incidence of cardiac malformations at relatively low levels of drinking water exposure (e.g., Johnson et al., 2005, 2003), while others reported no excess cardiac malformations at much higher oral (Fisher et al.,

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<sup>4</sup> The term “teratogenicity” refers only to malformations, which is one of several types of toxicity that may adversely affect a developing organism. The term “developmental toxicity” is the broader term used to describe the several types of toxicity and consequential adverse effects on a developing organism. The major manifestations of developmental toxicity include: 1) death of the developing organism, 2) structural abnormality (i.e., malformations), 3) altered growth, and 4) functional deficiency.

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2001) or inhalation (e.g., Carney et al., 2006) exposures. A number of methodological factors may have contributed to differences across study outcomes, such as the route of administration, test substance purity, test species or strain, timing of dosing or fetal evaluation, procedures used in dissecting and examining fetal hearts, statistical approaches applied to data evaluation, and generally uncharacterized inter-laboratory variation.

While some considered the epidemiologic and rodent data to be inadequate for providing evidence of TCE cardiac teratogenicity (Watson et al., 2006; Hardin et al., 2005), the NRC (2006) and SAB (2011) noted that there are relevant data from avian and in vitro mechanistic studies that provide additional support. For instance, studies in chick embryos reported consistent effects on cardiogenesis (e.g., hypoplastic cardiac cushions, reduced cardiac output, and septal and valvular alterations) when TCE, or equimolar concentrations of the oxidative metabolite trichloroacetic acid (TCA), was administered during critical stages of heart development (Rufer et al., 2010; Drake et al., 2006b; Drake et al., 2006a; Loeber et al., 1988). Some of the cardiac malformations reported in chicks are similar to those observed in rodent studies following in utero TCE exposures.

The events of cardiac morphogenesis in birds and mammals are similar; both involve mesenchymal cells that form endocardial cushion tissue with subsequent differentiation into septa and valvular structures in the adult heart (NRC, 2006). Thus, cultured embryonic chick atrioventricular canal cushion cells have been used as a widely accepted model to examine chemically induced disruptions in cardiac morphogenesis. In this model, TCE has inhibited endothelial separations and mesenchymal cell formation (Mishima et al., 2006; Boyer et al., 2000) and adhesive properties of endocardial cells (Hoffman et al., 2004), either of which could potentially result in septal or valvular malformations. Other TCE-induced effects observed in these systems that may have morphologic consequences in the developing heart include disruption of endothelial oxide synthetase, which has a role in endothelial cell proliferation (Ou et al., 2003), and interference with proteins involved in intercellular  $Ca^{2+}$  regulation, which may result in altered blood flow (Caldwell et al., 2010; Caldwell et al., 2008; Selmin et al., 2008; Collier et al., 2003).

Overall, the avian and in vitro data support the biological plausibility of TCE-induced cardiac teratogenesis and thus provide additional information to the epidemiologic and in vivo rodent data that suggest TCE induces cardiac teratogenicity (i.e., site concordance across species). Moreover, the mechanistic data support the possibility that multiple modes of action with different targets within the developing heart may be operant in eliciting cardiac malformations, which is consistent with the reported association between TCE and overall cardiac malformations in the absence of a strong association with any particular type of malformation. Finally, because the mechanistic data are specific to cardiac teratogenesis, the conclusions based on these data are limited to the endpoint of cardiac malformations only, and not applicable to more general developmental toxicity.

In light of these conclusions, the IRIS Toxicological Review of TCE used the only available study of cardiac malformations that was suitable for dose-response assessment (Johnson et al.,

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2003) to derive one of the candidate RfCs that supports the overall RfC (see Sections 4.8.3.3.2 and 5.1.2.8 of the IRIS Toxicological Review of TCE). Johnson et al. (2003) exposed animals to TCE via drinking water, so a physiologically based pharmacokinetic (PBPK) model (Chiu et al., 2009) was used to perform route-to-route extrapolation in the derivation of the candidate RfC. The EPA Science Advisory Board (SAB), in its independent peer review of the IRIS Toxicological Review of TCE, agreed with this approach, recommending that “The two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) should be considered the principal studies supporting the RfC” (SAB, 2011). Furthermore, because each of these are considered “principal studies,” each could support the RfC independently.

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**1.2. Summary Conclusion for Question 1: Are the data on TCE and cardiac malformations adequate to infer a site-concordant hazard from TCE specifically for this developmental endpoint rather than a general developmental toxicity hazard?**

Yes. The hazard conclusion with respect to cardiac malformations from the IRIS Toxicological Review of TCE was based on consideration of the weight of evidence from epidemiologic, rodent, avian, and in vitro data on this endpoint and reflects an evaluation of cardiac teratogenicity specifically and not of developmental toxicity in general. As a result, it is appropriate to use cardiac teratogenicity alone as the basis for considering less-than-chronic exposure durations for comparability to the IRIS TCE RfC.

## **2. COMPARABILITY TO THE RFC FOR TCE- INDUCED CARDIAC MALFORMATIONS FOR DIFFERENT EXPOSURE DURATIONS**

### **2.1. Background and Approach**

Given the inference of a site-concordant hazard from TCE for cardiac malformations, the next step is to determine whether sufficient evidence exists to specify the appropriate duration of exposure to consider when characterizing risk for this developmental effect using the RfC. As discussed in the Preface, the RfC is defined in the context of continuous exposure at a constant level over a chronic duration. However, if exposure levels are variable, even if average exposures over a longer duration are without appreciable risk, fluctuations leading to higher levels during a shorter window of susceptibility may be of concern. Therefore, the aim of this section is to analyze the “comparability to the IRIS TCE RfC” when different exposure durations are used as the basis for averaging exposures. Specifically, a number of exposure durations are evaluated as to whether ECs derived by averaging fluctuating exposure levels over that duration would yield a characterization of risk comparable to that derived for a constant, continuous, chronic exposure scenario.

#### **2.1.1. Exposure durations and developmental toxicity**

It is a well-established fact that a single exposure during a developmental window can in some cases cause an adverse developmental effect (U.S. EPA, 1991; Wilson, 1973). Chronic exposures can also cause adverse developmental effects, since they will include exposures during the relevant developmental window. Therefore, it is common practice is to consider exposure durations ranging from acute to chronic when developing reference values based on developmental effects (Solecki et al., 2010; U.S. EPA, 2002). However, although both acute and chronic exposures can cause an effect (i.e., a hazard exists), the level of response may not be the same, depending on the window of susceptibility, the extent to which the response depends on the cumulative (integrated) internal dose, and the toxicokinetics related to potential bioaccumulation of internal dose.

The comparability between single and repeated exposures has been investigated quantitatively to a limited degree. van Raaij et al. (2003) compared LOAELs and NOAELs for malformations (among other developmental effects) between single and repeated exposures for 10 compounds. Some substances showed little difference (<2-fold) between single and repeated dose LOAELs or NOAELs, while others showed clear (>3-fold) differences in which the repeated dose LOAELs or NOAELs were lower than single dose LOAELs or NOAELs. Similarly, Davis et al. (2009)

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compared developmental effects reported in single-dose studies with those reported in repeated-dose studies of the same compound using benchmark dose modeling. The study authors selected two representative compounds for which such data were available: butyl benzyl phthalate (short half-life of 6–7 hr) and tributyltin chloride (long half-life of 23–30 days), each of which was associated with multiple developmental effects, including organ malformations. As expected, their analysis revealed no qualitative differences between single- and repeated-dose studies in terms of the endpoints observed. However, they did find quantitative differences for these two compounds—specifically, that to elicit the same response level for a developmental effect, higher single-day exposures are required as compared to multi-day exposures, suggesting the importance of cumulative exposure (and presumably cumulative [integrated] internal dose) in these cases.

**2.1.2. Approach to analyzing the comparability to the IRIS TCE RfC for different exposure durations**

The purpose of this Chapter is to estimate the TCE exposure duration over which ECs may be averaged that would yield a characterization of risk comparable to that estimated for constant, continuous, chronic exposure scenario. A number of factors need to be considered when analyzing the comparability to the IRIS TCE RfC for different exposure durations, including the following:

- the duration of the window of susceptibility for TCE-induced cardiac malformations in humans;
- the extent to which the response depends on cumulative internal dose integrated over the window of susceptibility; and
- the toxicokinetics related to potential bioaccumulation of internal dose.

These factors are discussed in the following sections to inform conclusions as to the comparability to the IRIS TCE RfC for four different exposure durations: a chronic duration, nine months, three weeks, and one day.

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**2.2. The duration of the window of susceptibility for TCE-induced cardiac malformations in humans**

The window of susceptibility is the period of time over which toxicant exposure at the target site (internal dose) may cause adverse effects. By definition, exposures outside of the window of susceptibility cannot cause effects. Additionally, if exposure levels are variable over the duration of exposure, even if average exposures over a longer duration are without appreciable risk, fluctuations leading to higher levels during the window of susceptibility may be of concern. Therefore, when considering “comparability to the IRIS TCE RfC” for different exposure durations, it is essential to understand the duration of the window of susceptibility in humans for the effect of concern.

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In the case of TCE-induced cardiac malformations, four options are considered for the duration of the window of susceptibility in humans:

- a chronic duration;
- the duration of human pregnancy (nine months);
- the full duration of major human cardiac morphogenesis (three weeks); and
- one day during the period of major human cardiac morphogenesis.

These are discussed in sequence below.

**2.2.1. Chronic duration**

It is well-established that windows of susceptibility for developmental effects are less-than-chronic in duration. Therefore, the choice of a chronic duration (including a lifetime) for the window of susceptibility for TCE-induced cardiac malformations is not supported.

**2.2.2. The duration of human pregnancy (nine months)**

The experimental animals (rats) in the principal study (Johnson et al., 2003) were exposed to TCE from conception to gestation day 22 (the full length of gestation), with the critical effects in fetuses evaluated on the last day. Exposures were originally reported as concentrations in drinking water, and the point of departure in the IRIS Toxicological Review of TCE was based on daily oral doses in adult dams estimated by the authors, extrapolated to the inhalation route using a PBPK model on the basis of daily average maternal internal dose (see Section 5.1.3.1.3 of the IRIS Toxicological Review of TCE for additional discussion of the dose metric used).

One approach, therefore, would be to define the window of susceptibility for TCE-induced cardiac malformations as the human life stage that corresponds to the life stage during which the rat was exposed. Because the animals in the Johnson et al. (2003) study were exposed throughout pregnancy, the corresponding human life stage would be the developing embryo/fetus during human pregnancy. Under this approach, nine months would be used as the exposure duration for comparability to the RfC.

For developmental effects such as decreased weight or embryonic or fetal death, it may be difficult to clearly identify a susceptible period during gestation. In such cases, it may be difficult to specify a shorter duration as the developmental window. However, organ defects such as cardiac malformations are associated with shorter, more specific developmental windows during gestation, rather than the entire gestational period. Therefore, the choice of nine months as the window of susceptibility for TCE-induced developmental cardiac malformations is not supported, because it extends beyond the developmental window for major human cardiac morphogenesis.

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**2.2.3. The full duration of major human cardiac morphogenesis**

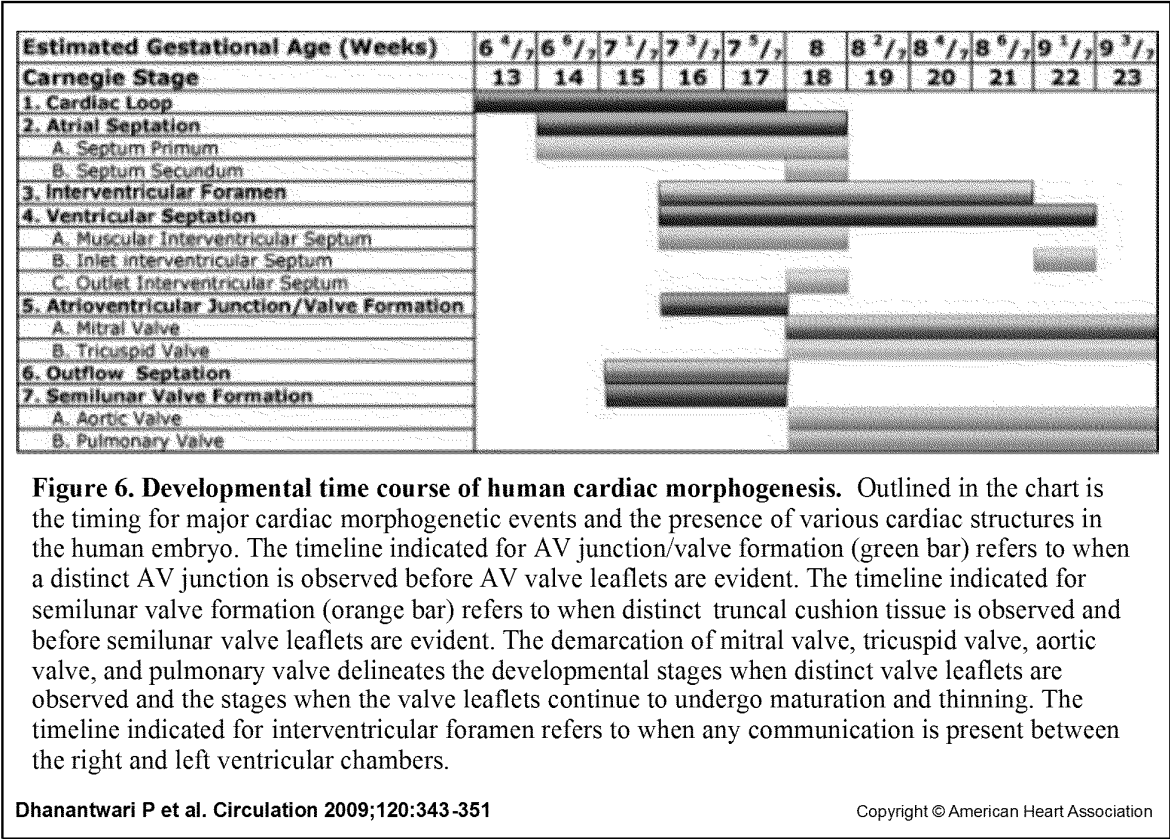
In humans, the most sensitive period for cardiac teratogenesis occurs during organogenesis, from approximately three weeks to about five or six weeks after conception (Sadler, 2000). During this period, several cardiac milestones occur, including development of the atrial, ventricular, and conotruncal septa. A recent study using advanced medical imaging has provided more detailed documentation of the major milestones of human cardiac morphogenesis (Dhanantwari et al., 2009). As shown in Figure 2-1, reproduced from this study, specific cardiac morphogenic events have been observed to occur over various times from estimated gestational ages 6<sup>4</sup>/<sub>7</sub> weeks to 9<sup>3</sup>/<sub>7</sub> weeks (equivalent to Carnegie Stages 13 to 23),<sup>5</sup> with different events occurring throughout this period. Moreover, Dhanantwari et al. (2009) state that “[t]hese stages encompass the developmental window during which all of the major milestones of cardiac morphogenesis can be observed.” These studies suggest a two- to three-week period as the window for major human cardiac organogenesis. More weight is given to a three-week period, as it is both consistent with the summary from Sadler (2000) and supported by the highly detailed study of Dhanantwari et al. (2009).

Apparent discrepancies in the precise timing of the developmental window between these studies are likely due, at least in part, to differences in the techniques used to determine the age of the embryo: Sadler (2000) used estimated time from conception for aging the embryo, whereas Dhanantwari et al. (2009) used estimated gestational age, which is calculated from the onset of the last menstrual period (LMP). However, the interval between LMP and conception can vary from 7 days to more than 25 days (IOM, 2007). When the estimated gestational age is adjusted to be consistent with the age since conception, the highly sensitive period is similar across both studies.

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<sup>5</sup> Note: Carnegie Stages (1-23) are used by embryologists to describe the apparent maturity of a vertebrate embryo based on external features.

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**Figure 2-1. Developmental time course of major human cardiac morphogenesis. Reproduced from Dhanantwari et al. (2009).<sup>5</sup>**

Several factors suggest that the window of susceptibility for TCE-induced cardiac malformations encompasses the entire period of major human cardiac morphogenesis, rather than only a part of it. First, as discussed in the IRIS Toxicological Review of TCE, the increased incidence of cardiac malformations reported by Johnson et al. (2003) constituted an aggregate increase in multiple types of cardiac malformations, rather than an increase in any one specific kind of malformation. Consequently, all major human cardiac morphogenic events are potentially relevant, and alteration of any of them can contribute to cardiac malformations. Additionally, the IRIS Toxicological Review of TCE concluded that multiple modes of action, involving multiple cell types and different types of TCE-induced alterations, are likely to be involved in causing cardiac malformations. It is plausible that these different modes of action lead to different types of malformations, each with different windows of susceptibility, in which case exposure over all of these multiple windows would be needed to elicit the full spectrum of types of malformations reported in the critical study and used in the derivation of the RfC. In this case, exposure during only one of these windows would therefore only elicit a subset of malformations, and therefore there would still be susceptibility outside of that window.

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In sum, the available scientific data support the choice of three weeks as the window of susceptibility for TCE developmental cardiac teratogenicity. Because the IRIS TCE RfC is based on the aggregate of multiple types of cardiac malformations, exposure during only part of the full three-week period would not fully cover the periods during which the heart may be susceptible to teratogenesis. Therefore, the three-week exposure duration, covering the full period of major human cardiac morphogenesis, encompasses the window necessary to elicit the full range, as opposed to only a subset, of TCE-induced cardiac malformations.

**2.2.4. One day during the period of major human cardiac morphogenesis**

Available data show the duration over which any one major cardiac morphogenic event occurs to be as short as 2 days in humans (as shown in Figure 2-1). It is a well-established fact that developmental effects may result from a single exposure. Therefore, another option for the window of susceptibility for TCE-induced cardiac malformations would be one day. However, as discussed above, several lines of evidence suggest that TCE can elicit multiple types of malformations in various cardiac structures that have varying morphogenic periods. Thus, exposure solely during a period shorter than three weeks would be expected to elicit only the specific kind(s) of malformation(s) associated with the morphogenic event(s) occurring during that time period. This is not to say that a one-day exposure would not pose a potential hazard, since a specific subset of malformations may be elicited by such an exposure. However, the full window over which humans may be susceptible to TCE-induced cardiac teratogenesis likely extends longer than one day. Therefore, the available scientific data do not support one day during the period of major human cardiac morphogenesis as the window of susceptibility.

**2.2.5. Conclusions with respect to the window of susceptibility for TCE-induced cardiac malformations**

The available scientific data support three weeks, approximately equal to the period of major human cardiac morphogenesis, as the window of susceptibility for TCE-induced developmental cardiac teratogenicity. Longer periods, including a chronic duration and nine months (corresponding to the duration of human pregnancy), are not supported, as they extend beyond the duration of major human cardiac morphogenesis. The shorter period of one day is not supported, because evidence suggests that multiple types of cardiac malformations may be elicited by TCE, and shorter durations would not cover the entire period over which humans may be susceptible to TCE-induced cardiac teratogenesis.

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**2.3. Extent to which response depends on cumulative internal dose integrated over the window of susceptibility**

As discussed in Section 2.1, available analyses suggest that acute exposure often, but not always, leads to lower responses as compared to repeated or chronic exposure at the same exposure level. In the absence of chemical-specific data, it is common practice to make the more

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protective assumption of equal responses for acute and repeated exposures with respect to developmental effects. This assumption is supported by the fact that it has been shown to be true in a number of cases.

In the case of TCE, however, several lines of evidence suggest that a one-day exposure would correspond to a lower level of response as compared to exposure over the full developmental window, and that therefore, the response depends more strongly on cumulative internal dose integrated over the entire window of susceptibility than on exposure during any single day. First, as discussed above, the increased incidence of cardiac malformations reported by Johnson et al. (2003) constituted an aggregate increase in multiple types of cardiac malformations rather than an increase in any one specific kind of malformation. Thus, shorter-term exposures above the RfC could lead to increased incidence of only a specific subset of malformations, so a higher exposure level would be necessary to elicit an incidence of effects comparable to an exposure over the full three-week period.<sup>6</sup> Additionally, the IRIS Toxicological Review of TCE concluded that multiple modes of action are likely to be involved in causing cardiac malformations, in which case exposure affecting all the different modes of action would be needed to elicit the full cardiac teratogenic response. Thus, exposure during a shorter window involving only one of these modes of action would only elicit a subset of malformations, with corresponding lower overall response. Therefore, though the possibility of differences in susceptibility at different time points within the window of susceptibility cannot be ruled out, the available scientific evidence supports the importance of cumulative internal dose integrated over the full three-week window of major human cardiac morphogenesis for TCE-induced cardiac teratogenesis.

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## **2.4. Toxicokinetics related to potential bioaccumulation.**

### **2.4.1. General background on the potential impact of bioaccumulation**

A basic premise of teratogenesis is that the fetal response is based on the internal dose during the critical developmental window for the effect. This implies that internal doses before and after the critical window are irrelevant, in the sense that they do not contribute to the dose-response relationship. Therefore, although the RfC is based on a chronic, constant, continuous exposure scenario, only the time period during which the internal dose overlaps with the critical

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<sup>6</sup> Specifically, the point of departure for this endpoint is based on a benchmark response level of 1% extra risk for fetal cardiac malformations. Therefore, the RfC represents the chronic exposure level that would likely be without a 1% extra risk for this endpoint in a sensitive individual, and is protective with respect to variability in the human population and uncertainties in the point of departure and in interspecies toxicodynamics. Based on the discussion in this section, it would be expected that a single-day exposure at the RfC would correspond to an exposure level that addresses a *less than* 1% extra risk in a sensitive individual. Conversely, it is expected that some single-day exposures above the RfC would correspond to an exposure level that addresses a 1% extra risk in a sensitive individual.

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window contributes to the response. However, depending on the toxicokinetics (TK) of the compound, exposures preceding the critical developmental window may also contribute to an internal dose during that window.

For instance, in the case of a compound with a long biological half-life relative to the duration of the developmental window (e.g., one that bioaccumulates), exposures substantially prior to the developmental window may also be relevant, because the internal dose may remain elevated long after exposure has ceased. Additionally, longer-term exposure at lower levels to substances that bioaccumulate can result in internal doses comparable to those from shorter-term exposures at higher levels. Such bioaccumulation would need to be addressed in the dose-response assessment, and the results could vary depending on the exposure duration of interest.

On the other hand, if a compound has a short biological half-life relative to the duration of the developmental window, then exposure and internal dose at any given time are highly correlated. In such cases, only exposure during the critical developmental window is relevant.

#### **2.4.2. Application to TCE internal dose**

As described in the IRIS Toxicological Review of TCE, TCE and its metabolites are completely eliminated within a few days of exposure in mice and rats. In humans, the parent compound and most of the metabolites are eliminated within a week of exposure, with TCA being completely eliminated over the course of about a month. Thus, the potential impact of bioaccumulation would not be expected to be large.

The potential impact of bioaccumulation of internal dose can be directly estimated by calculating human equivalent concentration (HEC)-based points of departure for various exposure durations with the Chiu et al. (2009) PBPK model used in the IRIS Toxicological Review of TCE. Specifically, the IRIS Toxicological Review calculated chronic HECs corresponding to the benchmark internal dose estimated from the Johnson et al. (2003) study, using daily oxidative metabolism as the internal dose metric. The chronic HECs can be compared to HECs calculated based on less-than-chronic exposure durations, using the same internal dose metric, as shown in Tables 2-1 and 2-2, for continuous and intermittent (occupational) exposure scenarios.

As expected, the impact of internal dose bioaccumulation is relatively small. For constant, continuous exposures for durations as short as one day, the HECs differ from those calculated for chronic exposure by less than 15%. For the intermittent (occupational) exposure scenarios examined, HECs differ by less than 10%. In both cases, the HECs predicted for an exposure duration of three weeks, corresponding to the full period of major human cardiac morphogenesis, differed from the chronic HECs by less than 2%.

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**Table 2-1. Comparison of TCE human equivalent concentrations (HECs) under constant, continuous exposure for different exposure durations**

| TCE Exposure Duration<br>(constant, continuous exposure) | Human Inhalation Concentration Equivalent to the Benchmark Dose Lower Confidence Limit for Cardiac Malformations ( $\mu\text{g}/\text{m}^3$ ) <sup>a</sup> |  |
|--|--|--|
|  | Median estimate  | Upper 99 <sup>th</sup> percentile estimate |
| Chronic (steady-state)                                   | 62   | 20   |
| 9 months (40 weeks)                                      | 62   | 20   |
| 3 weeks  | 63   | 20   |
| 1 day  | 71   | 21   |

<sup>a</sup>The internal dose metric of  $\mu\text{g}$  oxidized per day per  $[\text{kg body weight}]^{3/4}$  was selected as the basis for interspecies, intraspecies, and route -to-route extrapolation for the cardiac malformations endpoint in the IRIS Toxicological Review of TCE. The PBPK model was parameterized for human females. HECs are rounded to two significant figures.

**Table 2-2. Comparison of TCE human equivalent concentrations (HECs) under intermittent (occupational) exposure for different exposure durations**

| TCE Exposure Duration<br>(intermittent exposure<br>8 hr/day, 5 day/wk) | Human Inhalation Concentration Equivalent to the Benchmark Dose Lower Confidence Limit for Cardiac Malformations ( $\mu\text{g}/\text{m}^3$ ) <sup>a</sup> |  |
|--|--|--|
|  | Median estimate  | Upper 99 <sup>th</sup> percentile estimate |
| Chronic (steady-state)   | 62   | 20   |
| 9 months (40 weeks)  | 62   | 20   |
| 3 weeks  | 63   | 20   |
| 1 day  | 68   | 21   |

<sup>a</sup>The internal dose metric of  $\mu\text{g}$  oxidized per day per  $[\text{kg body weight}]^{3/4}$  was selected as the basis for interspecies, intraspecies, and route -to-route extrapolation for the cardiac malformations endpoint in the IRIS Toxicological Review of TCE. The PBPK model was parameterized for human females. Note that standard duration adjustments have been applied to the chronic HECs calculated from the PBPK model. Specifically, for chronic, nine month, and three week durations, an adjustment for 8/24 hours per day and 5/7 days per week has been applied; for the one day duration, an adjustment of 8/24 hours per day has been applied. For instance, the median HEC for the 1 day duration was calculated as a single, 8 hour exposure at  $203 \mu\text{g}/\text{m}^3$ , to which a duration adjustment of 8/24 was applied to derive the reported value of  $68 \mu\text{g}/\text{m}^3$ . HECs are rounded to two significant figures.

## **2.5. Synthesis for the evaluation of comparability to the IRIS TCE RfC for different exposure durations**

The analysis in this section focused on three factors that needed to be considered when analyzing different exposure durations for comparability to the IRIS TCE RfC: (a) the duration of the window of susceptibility for TCE-induced cardiac malformations in humans; (b) the extent to which the response depends on cumulative internal dose integrated over the window of susceptibility; and (c) the toxicokinetics related to potential bioaccumulation of internal dose. These factors are the basis of the following conclusions.

### **2.5.1. Chronic duration**

The available scientific data support the conclusion that ECs derived by averaging fluctuating exposure levels over a chronic duration may not yield a characterization of risk comparable to that derived for a constant, continuous, chronic exposure scenario. This is due to the fact that the window of susceptibility for TCE-induced cardiac malformations is much shorter than a chronic duration. Therefore, even if overall average exposures over this duration are at or below the IRIS TCE RfC, there may be appreciable risk for cardiac malformations due to fluctuations above the RfC during the window of susceptibility. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a chronic duration would be expected to underestimate the level of human health concern.

### **2.5.2. Duration of human pregnancy (nine months)**

Similarly, the available scientific data support the conclusion that ECs derived by averaging fluctuating exposure levels over the nine-month duration of human pregnancy may not yield a characterization of risk comparable to that derived for constant, continuous, chronic exposure scenario. This is due to the fact that the window of susceptibility for TCE-induced cardiac malformations is shorter than the full duration of human pregnancy. Therefore, even if overall average exposures over this duration are at or below the IRIS TCE RfC, there may be appreciable risk for cardiac malformations due to fluctuations above the RfC during the window of susceptibility. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a nine-month duration would be expected to underestimate the level of human health concern.

### **2.5.3. The full duration of major human cardiac morphogenesis (three weeks)**

The available scientific data support the conclusion that ECs derived by averaging fluctuating exposure levels over the three-week duration of major human cardiac morphogenesis would yield a characterization of risk comparable to that derived for a constant, continuous, chronic exposure scenario. This is due to the evidence presented above that:

- the period of major human cardiac morphogenesis is approximately three weeks;

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- several lines of evidence suggest that the response depends on cumulative internal dose integrated over the window of susceptibility;
- the impact of toxicokinetics on bioaccumulation of internal dose for this exposure duration is relatively small.

Using a three-week duration averaging period for fluctuating exposures assumes that different time points within the developmental window contribute equally to the overall response, and as such, the response is a function of cumulative internal dose integrated over that time period. This assumption is reasonable for the reasons discussed in this section and given the absence of data on whether specific events within the developmental window are more or less susceptible to disruption by TCE. Therefore, intermittent exposures (e.g., occupational scenarios) could be evaluated on the basis of a time-weighted average over the developmental window.<sup>7</sup> However, it is not known whether a specific subset of malformations is more likely to result from TCE exposure, and if therefore, there are shorter periods within the three-week period of major human cardiac morphogenesis that are more sensitive to TCE-induced cardiac teratogenicity. Specifically, as opposed to all time points within the three-week period contributing equally to the overall response, it is possible that some shorter periods contribute more, while other periods contribute less. Thus, some uncertainty remains in the comparability of a three-week duration with the IRIS TCE RfC. Overall, however, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a three-week duration would be expected to neither underestimate nor overestimate the level of human health concern.

**2.5.4. One day during the period of major human cardiac morphogenesis**

As discussed above, the available scientific data lend greater support to three weeks as the duration over which ECs can be averaged to yield a characterization of risk comparable to that derived for constant, continuous, chronic exposure scenario. However, the possibility of a shorter window, including one day, also being of human health concern cannot be ruled out. Specifically, uncertainties remain in whether there are shorter periods that are more sensitive than the average sensitivity over the entire three-week period of major human cardiac morphogenesis. One day's exposure at the RfC would likely be without appreciable risk, and protective of the possibility that one particular day during the period of major human cardiac morphogenesis is more sensitive to TCE-induced cardiac teratogenesis. However, to reach the same level of response, a higher one-day

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<sup>7</sup> However, for extremely high levels of fluctuations in exposure over three-week periods, it could be more appropriate to consider the exposure scenario as reflecting a shorter duration. For instance, in an extreme example where exposure occurs only on a single day with no exposure on the other 20 days of the three-week period, it would be more appropriate to consider this an acute exposure scenario, rather than to calculate a three-week time-weighted-average exposure concentration, which would be 21-times lower than the single day exposure.

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EC would be expected to be needed as compared to a three-week EC. Therefore, it would be expected that a one-day EC higher than the RfC could also be considered likely to be without appreciable risk, although data are inadequate to estimate how much higher such a level might be. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a one-day duration would be expected to overestimate the level of human health hazard concern.

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**2.6. Summary Conclusion for Question 2: What is the most appropriate exposure duration for comparability to the RfC for TCE-induced cardiac malformations?**

- **The available scientific data do not support using either a chronic or nine-month period as the exposure duration for comparability to the RfC because these exposure durations exceed the window of susceptibility.** If one of these durations were used for calculating the time-weighted average EC for comparison with the RfC, the resulting risk characterization would not be expected to be comparable to the risk characterization based on a constant, continuous, chronic exposure scenario. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over such durations would be expected to underestimate the level of human health concern.
- **The available scientific data supports using the three-week period of major human cardiac morphogenesis as the exposure duration for comparability to the RfC.** In particular, this duration corresponds to the window of susceptibility, and evidence suggest that the the response depends on cumulative internal dose integrated over the full window of susceptibility. Additionally, for this exposure duration, the impact of toxicokinetics on bioaccumulation of internal dose is relatively small. If a three-week period were used for calculating the time-weighted average EC for comparison with the RfC, the resulting risk characterization is expected to be comparable to the risk characterization based on a constant, continuous, chronic exposure scenario. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a three-week duration would be expected to neither underestimate nor overestimate the level of human health concern.
- **The available scientific data do not rule out the possibility that one day of exposure during the three-week period of major human cardiac morphogenesis might be of concern.** In particular, if a one day duration were used for calculating the time-weighted average EC for comparison with the RfC, the resulting risk characterization would be protective of the possibility that one particular day during the period of major human cardiac morphogenesis is more sensitive than average to TCE-induced cardiac teratogenesis. However, to reach the same level of response reflected by the RfC, a higher one-day EC would be expected to be needed as compared to a three-week EC. Therefore, it would be expected that a one-day EC higher than the RfC could be considered likely to be without appreciable risk, although data are inadequate to estimate how much higher such a level might be. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a one-day duration would be expected to overestimate the level of human health concern.

### 3. SUMMARY

This Addendum discusses how the available data on TCE, cardiac malformations, and cardiac development may inform the appropriate duration for derivation of ECs for comparison with the IRIS TCE RfC. Therefore, the goal of this Addendum is to estimate the “exposure duration for comparability to the RfC,” which is defined as the exposure duration over which ECs may be averaged that would yield a characterization of risk comparable to that estimated for a constant, continuous, chronic exposure scenario. Determining what exposure duration is most comparable to a constant, continuous, chronic exposure scenario depends on a number of different considerations, analyzed in Chapters 1-2. The results of this analysis are as follows:

- 1) Are the data on TCE and cardiac malformations adequate to infer a site-concordant hazard from TCE specifically for this developmental endpoint rather than a general developmental toxicity hazard?

Yes. The hazard conclusion with respect to cardiac malformations from the IRIS Toxicological Review of TCE was based on consideration of the weight of evidence from epidemiologic, rodent, avian, and in vitro data on this endpoint and reflects an evaluation of cardiac teratogenicity specifically and not of developmental toxicity in general.

- 2) What is the most appropriate exposure duration for comparability to the RfC for TCE-induced cardiac malformations?

- **The available scientific data do not support using either a chronic or nine-month period as the exposure duration for comparability to the RfC.** These exposure durations exceed the window of susceptibility, and a characterization of risk based on ECs derived by averaging fluctuating exposure levels over such durations would be expected to underestimate the level of human health concern.

- **The available scientific data support using the three-week period of major human cardiac morphogenesis as the exposure duration for comparability to the RfC.** A characterization of risk based on ECs derived by averaging fluctuating exposure levels over a three-week duration would be expected to neither underestimate nor overestimate the level of human health concern.

- **The available scientific data do rule out the possibility that one day of exposure during the three-week period of major human cardiac morphogenesis might be of concern.** However, to reach the same level of response reflected by the RfC, a higher one-day EC would be expected to be needed as compared to a three-week EC. Thus, a characterization of risk based on ECs derived by averaging fluctuating exposure levels over a one-day duration would be expected to overestimate the level of human health concern.

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***Addendum to the Toxicological Review of Trichloroethylene:  
Comparing Less-than-Chronic Exposure Concentration Estimates with the RfC***

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